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Applicant:	Wagner et al.	Examiner:	P. Gambel
Serial No.:	08/948,393	Art Unit:	1644
Filing Date:	October 10, 1997		
For:	METHOD FOR TREATING AND PREVENTING ATHEROSCLEROSIS		

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to the Commissioner for Patents, Washington, D.C. 20231 on 3/12/03

Patricia McKenney

BOX AMENDMENT
COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

Dear Sir:

DECLARATION UNDER 37 CFR 1.131

We, Denisa D. Wagner and Robert C. Johnson, declare and state as follows:

1. We are the applicants of the above-identified patent application, and the co-inventors of the subject matter disclosed and claimed therein.

2. We are familiar with the present claims of the above-identified application, which are directed to methods for treating or inhibiting atherosclerosis in a mammal by administering an agent that inhibits an interaction between P-selectin and PSGL-1 and E-selectin and a ligand of E-selectin., e.g. PSGL-1 (P-selectin glycoprotein ligand-1), soluble forms of PSGL-1, fragments

of PSGL-1 and mimetics of PSGL-1. As originally conceived, our invention embraced a broad range of P-selectin inhibitors, such as inhibitory proteins, peptides, glycoproteins, carbohydrates, antibodies and chimeric constructs.

3. We conceived the claimed invention at least as early as 1988, and coupled with due diligence from a time prior to November 16, 1992, reduced the claimed in invention to practice at least as early as May 6, 1994.

4. Exhibit A is a copy of a page showing a note authored by co-inventor Denisa Wagner in 1988. The notes shown in the Exhibit were recorded by Dr. Wagner during the conference of the American Heart Association held in 1988, and were written on the last page of the program booklet next to a listing of meetings to be held in 1989. The note on the bottom right hand side of the page states that

> Macrophages (Mφ) eat bits of activated platelets. ELAM-1 = Padgem. Do monocytes bind to Padgem on platelets. Padgem is an opsomizing agent to get rid of debris of platelets.

The term "Padgem" here refers to P-selectin and the term "ELAM-1" refers to E-selectin (Endothelial Leukocyte Adhesion Molecule). In 1988, E-selectin was known to mediate endothelial binding to leukocytes. We conceived that there is a functional relationship between E-selectin and P-selectin, and that P-selectin mediates the binding of platelets to macrophages (leukocytes implicated in atherosclerosis).By binding to Padgem, the macrophages are "eating" bits of activated platelets, thereby increasing the fat (lipid) content of the macrophages, and promoting their conversion into foam cells (macrophage cells with a "foamy" appearance due to the presence of lipids that act as precursors for atherosclerotic plaque). Exhibit A thus demonstrates that we had identified a role for P-selectin and E-selectin in some of the key pathological events involved in atherosclerosis, e.g. macrophage binding to P-selectin on platelets, from a time well before November 16, 1992.

5. Exhibit B, also written by Dr. Wagner, describes an experiment we conceived on February 28, 1992. Exhibit B states:

> Breed P-selectin deficient mouse with a mouse strain that develops atherosclerosis. See if it (atherosclerosis) can be prevented.

According to this proposed experiment, a mouse deficient in P-selectin would be bred with a mouse strain that develops atherosclerosis to determine whether atherosclerosis can be prevented. In other words, we conceived that if P-selectin/ligand binding and/or E-selectin/ligand binding could be inhibited *in vivo* in a mammal, the atherosclerotic lesions could be reduced or inhibited. In order to complete this experiment, we understood that it would first be necessary to prepare a P-selectin knock-out mouse, and breed this mouse with mouse strains susceptible to atherosclerosis. It is known that mice are generally resistant to developing atherosclerosis. The mouse strain most susceptible to developing atherosclerosis is the C57 black mouse. But the C57 mouse must still be fed a high lipid diet to observe any meaningful development of atherosclerosis.

6. Exhibit C, also written by Dr. Wagner, describes a proposal we conceived on March 2, 1992, to study the role of the P-selectin in atherosclerosis by developing a suitable mouse model, and feeding the P-selectin deficient mice (mutants) and control wild-type mice (P-selectin positive) with a lipid diet. The, formation of atherosclerotic lesions in the mice would be studied and characterized. Exhibit C states, on page 5:

> Study the role of P-selectin in atherosclerosis by feeding P-selectin deficient and P-selectin positive mice a lipid diet. Study the formation of atherosclerotic lesions in mice.

Page 5 of Exhibit C also poses the question whether von Willebrand (vW) disease pigs may be resistant to atherosclerosis because of a lack of P-selectin. P-selectin is stored in granules containing vW factor, and these granules are absent in vW disease.

7. At a time prior to November 16, 1992, we undertook to prepare a mouse model for subsequent testing. The mouse model took at least 4 years to prepare, and was completed on or about September 13, 1993. In order to prepare the mouse model, we used a knock-out mouse deficient in P-selectin and back-crossed this mouse with C57 black mice. In order to be sure that the resulting mutant mouse would be susceptible to atherosclerotic lesion development, we

decided to breed 4 generations of mice, with each generation being more susceptible to atherosclerosis. First we developed a P-selectin deficient mouse. Then we bred the P-selectin deficient mouse with a C57 black mouse. Finally, we bred the offspring of the first breeding with another C57 black mouse, and so on for a total of 4 back-cross breedings. We reasoned that the fourth generation would be suitable for evaluation. It took us about 3 years to make a P-selectin deficient mouse, and another year to complete the back-crossing process with the C57 black mice. This work was laborious and continuous, and consumed a large amount of our time and effort. Although the general technology for creating mouse models had been developed by others, we were the first to develop a P-selectin-deficient mouse model. We diligently worked on successfully constructing such a model, and verifying the correct properties and characteristics of the mutant mouse by about September 13, 1993.

8. After the preparation of the mutant mouse deficient in P-selectin on the C57 black background, we promptly commenced feeding the mice (control and experimental) a diet high in lipids. The experimental and control mice were fed a lipid diet for approximately eight months prior to sacrificing the animals and recording the data, This took approximately 8 months since even the C57 black mice are somewhat resistant to the formation of atherosclerosis. Immediately thereafter, we sacrificed the animals and evaluated them for the size and character of atherosclerotic lesions. We prepared the table enclosed as Exhibit D on May 6, 1994. The table in Exhibit D shows the size of atherosclerotic lesions in P-deficient (mutant) mice compared to wild type mice as controls. These results demonstrate a reduction in the size of atherosclerotic lesions in P-selectin deficient mice. Based on these results we concluded that inhibitors of P-selectin/ligand binding and/or E-selectin/ligand binding would be useful for the treatment or inhibition of atherosclerosis, and this constitutes an actual reduction to practice of the claimed invention.

9. From the above information, we deduced that inhibitors of P-selectin and/or E-selectin could be used to treat atherosclerosis in mammals based on the role of P-selectin and/or E-selectin on the pathogenesis of atherosclerosis as presently claimed in the above-identified application. We further believe that the above information constitutes evidence the claimed

invention was conceived prior to November 16, 1992, and diligently reduced to practice at least as early as the actual reduction to practice date of May 6, 1994.

We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

2/25/2003
Date

Denisa D. Wagner

3-6-03
Date

Robert C. Johnson

EXHIBIT A
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AMERICAN HEART ASSOCIATION CME OFFERINGS
1989 Highlights

For information contact the American Heart Association, Scientific Sessions, 7320 Greenville Avenue, Dallas, Texas 75231.

***SCIENTIFIC CONFERENCE ON MEMBRANE EVENTS AND INTRACELLULAR SIGNALLING IN THE CARDIOVASCULAR SYSTEM**
Waikoloa, Hawaii
AHA Council on Basic Science and the Japanese Heart Foundation
January 7-11, 1989
Conference Chairman: James T. Stull, PhD

14TH INTERNATIONAL JOINT CONFERENCE ON STROKE AND CEREBRAL CIRCULATION
San Antonio, TX
AHA Council on Stroke
February 9-11, 1989
Conference Chairman: Vladimir C. Hachinski, MD

SCIENTIFIC CONFERENCE ON CORONARY ATHEROSCLEROSIS AND THROMBOSIS
Keystone, CO
AHA Councils on Circulation, Atherosclerosis, Thrombosis, and Clinical Cardiology
February 22-25, 1989
Conference Chairman: Paul J. Cannon, MD

2ND INTERNATIONAL CONFERENCE ON PREVENTIVE CARDIOLOGY AND THE ANNUAL MEETING OF THE AHA COUNCIL ON EPIDEMIOLOGY
Washington, DC
AHA Council on Epidemiology
June 18-22, 1989
Conference Chairman: Jeremiah Stamler, MD

***15TH TEN-DAY SEMINAR ON THE EPIDEMIOLOGY AND PREVENTION OF CARDIOVASCULAR DISEASES**
Tahoe City, CA
AHA Council on Epidemiology
July 30-August 12, 1989
Conference Chairman: Darwin R. Labarthe, MD, PhD

43RD ANNUAL FALL CONFERENCE AND SCIENTIFIC SESSIONS OF THE COUNCIL FOR HIGH BLOOD PRESSURE RESEARCH
Cleveland, OH
AHA Council for High Blood Pressure Research
September 26-29, 1989
Conference Chairman: Allen W. Cowley, Jr, PhD

62ND SCIENTIFIC SESSIONS
New Orleans, LA
AHA Scientific Councils
November 13-16, 1989
Conference Chairman: Michael R. Rosen, MD

*Limited attendance

#23!? makes these vesicles are these some of Padgem ?

do plt release by ϕ blocks w 125β inhibit B/β 15-42

put flow through of ϕ column to on to fibrin column. ϕ rec. should be reduced. control do it reverse go back to fibrin clots does after reacting w fibrin the receptor get phosphorylated

Put on column EC grown in *Phosphate I stimulation w fibrin, EDTA elute If this works to #23 release - ϕ incubation etc.
Do x-linking - it will work

Add ϕ to all lysate put on fibrin column. ϕ should inhibit IIB IIIa-like binding but fibrin specific b. should not be affected !!

elute w RGD, b. to fibrin may not be through RGD or elute w γ pept.

see if severe vWd plts plts have Padgem

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mAb cat bits of activated plts ELAM-1 ! = Padgem

do monocytes b. to Padgem on plts
Padgem is an opsonizing agent to get rid of depress ↓ plts

EXHIBIT B
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feb. 28/92

Breed OP-sel mouse with a mouse strain that develops atherosclerosis see if it can be prevented.

Projects for Bob

3/2/92

Prepare antibodies to P-S. cytoplasmic tail (polyclonal). Do they recognise other granular proteins → clone them

Schaffhausen: Abs to SH2 domain of PDGF rec. binds to other prot. containing this homologous domain

Role of dibasic cleavage site in targeting to storage granules

Insulin c-DNA is available that has both sites mutated. When expressed in AtT-20 cells will it be stored?

Randy Kaufman has a protein inhibitor of PACE (and likely related enzymes. If could be transfected into cells and see if storage is prevented (ACTH, vWf etc).

In endothelial cells that do not express vWf — what happens to P-sel

a) culture EC in the presence

Role of vicinal cysteines in integrins matrix assembly?

Targeting of P-selectin in yeast

Is there a storage compartment in yeast use yeast secretion mutants and clathrin ⊖ cells to find the cellular machinery responsible for targeting of transmembrane proteins.

In the absence of vWf in EC, what happens to P-sel?

a) use vW disease pigs EC
does our Ab x-react?
disadvantage: availability
advantage - normal platelets: vWd pts

b) use #UVEC grown in the presence of antisense oligos to vWf
→ inhibit vWf synthesis
see where P-sel is and if it can be transported to cell surface
+/- secretagogues

vW disease pigs are resistant to atherosclerosis. Is this an effect of vWf (lack of) or P-selectin absence?

Study role of P-S. in atherosclerosis
by feeding ⊖ P-S and ⊕ P-S lipid diet to mice → formation of atherosclerotic lesions

5/6/94 Denise Boos

	Genotype	Score
271	Mut	−
278	WT	++ 2.7
279	Mut	−
137	Mut	−
119	WT	+ 0.5
548 E	Mut	+ 0.2
269	WT	++
270	Mut	+ small
40	WT	+++
34	Mut	−
35	WT	+
19	WT	+ (Maybe 2+)
20	WT	2+
268	WT	+++
42	Mut	−
106	WT	3+++
18	Mut	−
89	WT	−

Rachelle A. Rosenbaum 5/9/94
City of Boston
Suffolk County
MY COMMISSION EXPIRES JUNE 6, 1997
a true copy of the original

** See when it is deep lesion + not raised it is not a lesion. #34

#19 Must be positive about lesion.

Not good

EXHIBIT D
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